FIG._1

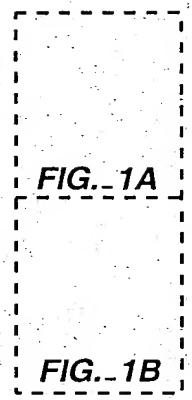

FIG._1A

SEQ ID NO: 1

Nucleotide Sequence Tankyrase Homologue isotype1

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TAGATGTGGGAAAGGAATTCTAGCAAGAGTTTTGTCACTGTATCATAAGGTTGTGATTTACATATTTAA
GTTTTATACTTTGAACATCTGAAAATGTATACATACTAAATATGCAGAACTCTATTGTAGAGTGAGAAA
CATTTGAACTTTGAGCTTTCAGTCACTTATTTTGTATTCTTTCTTTGAGGTTAGCAGTAGTACCACCCA
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GAAAGACGTAGTTGAATATTTGCTTCAGAATGGTGCAAGTGTCCAAGCACGTGATGATGGGGGCCTTAT
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CCCCAATGCTCGAGATAATTGGAATTATACTCCTCTCCATGAAGCTGCAATTAAAGGAAAGATTGATGT
TTGCATTGTGCTGTTACAGCATGGAGCTGAGCCAACCATCCGAAATACAGATGGAAGGACAGCATTGGA
TTTAGCAGATCCATCTGCCAAAGCAGTGCTTACTGGTGAATATAAGAAAGATGAACTCTTAGAAAGTGC
CAGGAGTGGCAATGAAGAAAAAATGATGGCTCTACTCACACCATTAAATGTCAACTGCCACGCAAGTGA
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GCAACATGGAGCTGATGTCCATGCTAAAGATAAAGGTGATCTGGTACCATTACACAATGCCTGTTCTTA
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ACTTCTTGTTAAACATGGAGCAGTAGTTAATGTAGCTGATTTATGGAAATTTACACCTTTACATGAAGC
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GTCTTTCCTGCAGTTCAGTGCAATGAAAATGGCACATTCTCCTCCAGGTCATCACTCAGTCACTGGTAG
GCCCAGTGTAAATGGCCTAGCATTAGCTGAATATGTTATTTACAGAGGAGAACAGGCTTATCCTGAGTA
TTTAATTACTTACCAGATTATGAGGCCTGAAGGTATGGTCGATGGATAAATAGTTATTTTAAGAAACTA
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AA

FIG._1B

FIG._2

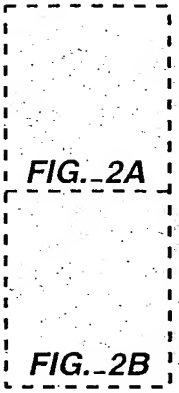

FIG._2A

SEQ ID NO: 2

Nucleotide Sequence Tankyrase Homologue isotype2

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CCCATGGGACTGCGCCGGATCCGGTGACAGCAGGGAGCCAAGCGGCCCGGGCCCTGAGCGCGTCTTCTC
CGGGGGGCCTCGCCCTCCTGCTCGCGGGGCCGGGGCTCCTGCTCCGGTTGCTGGCGCTGTTGCTGGCTG
TGGCGGCGGCCAGGATCATGTCGGGTCGCCGCTGCGCCGGCGGGGGAGCGGCCTGCGCGAGCGCCGCGG
CCGAGGCCGTGGAGCCGGCCGCCCGAGAGCTGTTCGAGGCGTGCCGCAACGGGGACGTGGAACGAGTCA
AGAGGCTGGTGACGCCTGAGAAGGTGAACAGCCGCGACACGGCGGGCAGGAAATCCACCCCGCTGCACT
TCGCCGCAGGTTTTGGGCGGAAAGACGTAGTTGAATATTTGCTTCAGAATGGTGCAAATGTCCAAGCAC
GTGATGATGGGGGCCTTATTCCTCTTCATAATGCATGCTCTTTTGGTCATGCTGAAGTAGTCAATCTCC
TTTTGCGACATGGTGCAGACCCCAATGCTCGAGATAATTGGAATTATACTCCTCTCCATGAAGCTGCAA
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GACATTATGAAGTTGCAGAACTTCTTGTTAAACATGGAGCAGTAGTTAATGTAGCTGATTTATGGAAAT
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TAGCAGCTGGTTATAATAATTTAGAAGTTGCAGAGTATTTGTTACAACACGGAGCTGATGTGAATGCCC
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AACAGGCTTATCCTGAGTATTTAATTACTTACCAGATTATGAGGCCTGAAGGTATGGTCGATGGATAAA
TAGTTATTTTAAGAAACTAATTCCACTGAACCTAAAATCATCAAAGCAGCAGTGGCCTCTACGTTTTAC
TCCTTTGCTGAAAAAAAAAAA

FIG._2B

SEQ ID NO: 3

Amino Acid Sequence Tankyrase Homologue isotype1

GFGRKDVVEYLLQNGASVQARDDGGLIPLHNACSFGHAEVVNLLLRHGADPNARDNWNYTPLHEAAIKG
KIDVCIVLLQHGAEPTIRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSGNEEKMMALLTPLNVNC
HASDGRKSTPLHLAAGYNRVKIVQLLLQHGADVHAKDKGDLVPLHNACSYGHYEVTELLVKHGACVNAM
DLWQFTPLHEAASKNRVEVCSLLLSYGADPTLLNCHNKSAIDLAPTPQLKERLAYEFKGHSLLQAAREA
DVTRIKKHLSLEMVNFKHPQTHETALHCAAASPYPKRKQICELLLRKGANINEKTKEFLTPLHVASEKA
HNDVVEVVVKHEAKVNALDNLGQTSLHRAAYCGHLQTCRLLLSYGCDPNIISLQGFTALQMGNENVQQL
LQEGISLGNSEADRQLLEAAKAGDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYNRVSVVEYLLQHG
ADVHAKDKGGLVPLHNACSYGHYEVAELLVKHGAVVNVADLWKFTPLHEAAAKGKYEICKLLLQHGADP
TKKNRDGNTPLDLVKDGDTDIQDLLRGDAALLDAAKKGCLARVKKLSSPDNVNCRDTQGRHSTPLHLAA
GYNNLEVAEYLLQHGADVNAQDKGGLIPLHNAASYGHVDVAALLIKYNACVNATDKWAFTPLHEAAQKG
RTQLCALLLAHGADPTLKNQEGQTPLDLVSADDVSALLTAAMPPSALPSCYKPQVLNGVRSPGATADAL
SSGPSSPSSLSAASSLDNLSGSFSELSSVVSSSGTEGASSLEKKEVPGVDFSITQFVRNLGLEHLMDIF
EREQITLDVLVEMGHKELKEIGINAYGHRHKLIKGVERLISGQQGLNPYLTLNTSGSGTILIDLSPDDK
EFQSVEEEMQSTVREHRDGGHAGGIFNRYNILKIQKVCNKKLWERYTHRRKEVSEENHNHANERMLFHG
SPFVNAIIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPVHKDRSCYICHRQLLFCRV
TLGKSFLQFSAMKMAHSPPGHHSVTGRPSVNGLALAEYVIYRGEQAYPEYLITYQIMRPEGMVDG

FIG._3

SEQ ID NO: 4

Amino Acid Sequence Tankyrase Homologue isotype2

RCSARRGAAGGQGAQRGARVGAAHGTAPDPVTAGSQAARALSASSPGGLALLLAGPGLLLRLLALLLAV
AAARIMSGRRCAGGGAACASAAAEAVEPAARELFEACRNGDVERVKRLVTPEKVNSRDTAGRKSTPLHF
AAGFGRKDVVEYLLQNGANVQARDDGGLIPLHNACSFGHAEVVNLLLRHGADPNARDNWNYTPLHEAAI
KGKIDVCIVLLQHGAEPTIRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSGNEEKMMALLTPLNV
NCHASDGRKSTPLHLAAGYNRVKIVQLLLQHGADVHAKDKGDLVPLHNACSYGHYEVTELLVKHGACVN
AMDLWQFTPLHEAASKNRVEVCSLLLSYGADPTLLNCHNKSAIDLAPTPQLKERLAYEFKGHSLLQAAR
EADVTRIKKHLSLEMVNFKHPQTHETALHCAAASPYPKRKQICELLLRKGANINEKTKEFLTPLHVASE
KAHNDVVEVVVKHEAKVNALDNLGQTSLHRAAYCGHLQTCRLLLSYGCDPNIISLQGFTALQMGNENVQ
QLLQEGISLGNSEADRQLLEAAKAGDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYNRVSVVEYLLQ
HGADVHAKDKGGLVPLHNACSYGHYEVAELLVKHGAVVNVADLWKFTPLHEAAAKGKYEICKLLLQHGA
DPTKKNRDGNTPLDLVKDGDTDIQDLLRGDAALLDAAKKGCLARVKKLSSPDNVNCRDTQGRHSTPLHL
AAGYNNLEVAEYLLQHGADVNAQDKGGLIPLHNAASYGHVDVAALLIKYNACVNATDKWAFTPLHEAAQ
KGRTQLCALLLAHGADPTLKNQEGQTPLDLVSADDVSALLTAAMPPSALPSCYKPQVLNGVRSPGATAD
ALSSGPSSPSSLSAASSLDNLSGSFSELSSVVSSSGTEGASSLEKKEVPGVDFSITQFVRNLGLEHLMD
IFEREQITLDVLVEMGHKELKEIGINAYGHRHKLIKGVERLISGQQGLNPYLTLNTSGSGTILIDLSPD
DKEFQSVEEEMQSTVREHRDGGHAGGIFNRYNILKIQKVCNKKLWERYTHRRKEVSEENHNHANERMLF
HGSPFVNAIIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPVHKDRSCYICHRQLLFC
RVTLGKSFLQFSAMKMAHSPPGHHSVTGRPSVNGLALAEYVIYRGEQAYPEYLITYQIMRPEGMVDG

FIG._4

Schematic Presentation of Dominant Negative Mutants for Tankyrase Homologue

Dominant Negative Mutants

Truncation: 429ΔC- of the C-terminal catalytic domain – truncation of the catalytic domain of PARP acts as a dominant negative when overexpressed *in vivo* (Oncogene 1999 Nov 25; 18(50):7010-5)

Point mutant: E945AΔC- conserved residue in PARP domain, thought to be important in NAD+ binding

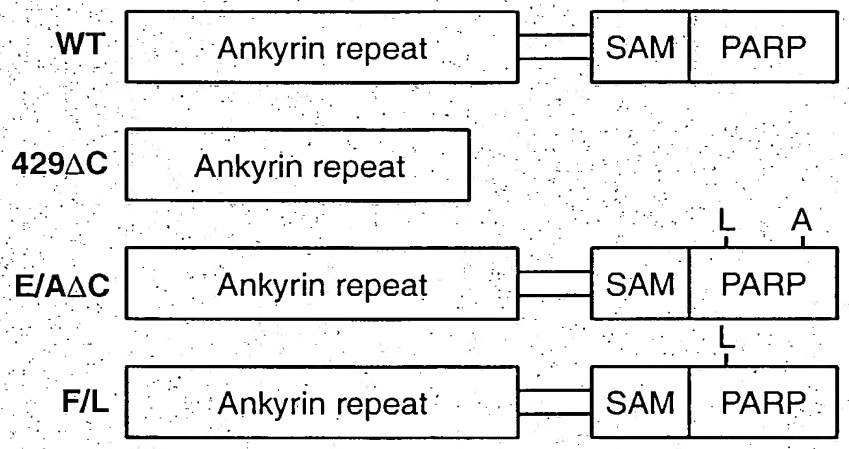


FIG._5

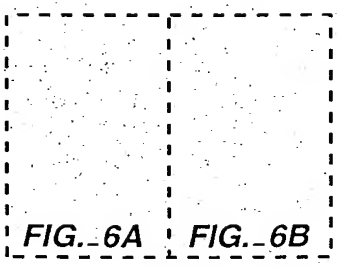


FIG._6

Cell Cycle Analysis of A549 Cells Infected With GFP-fused Wild Type and Mutant Tankyrase Homologue

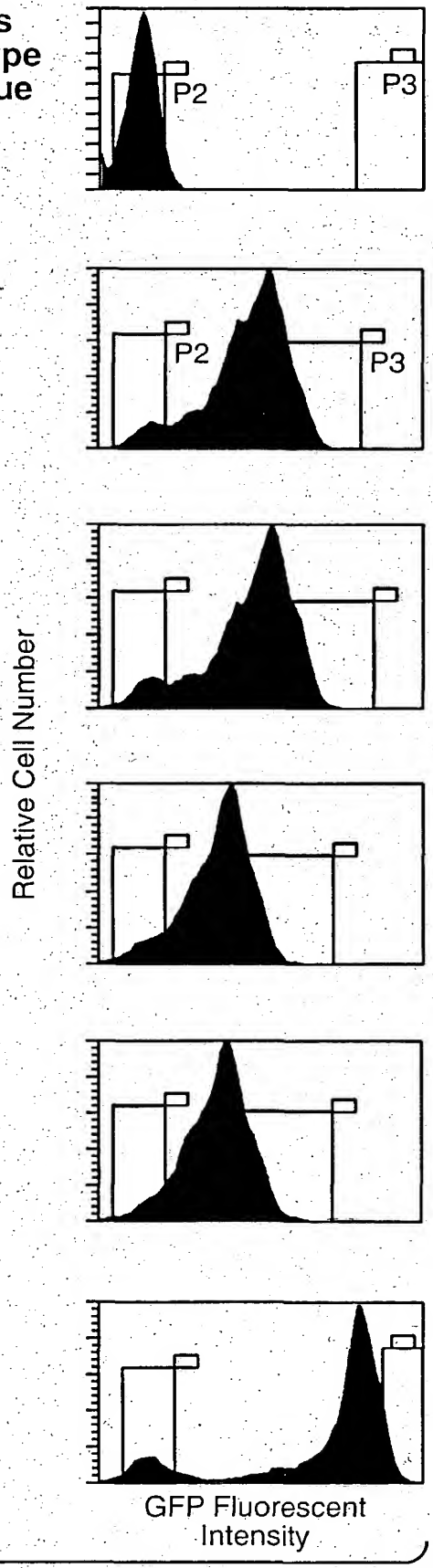


FIG 6A

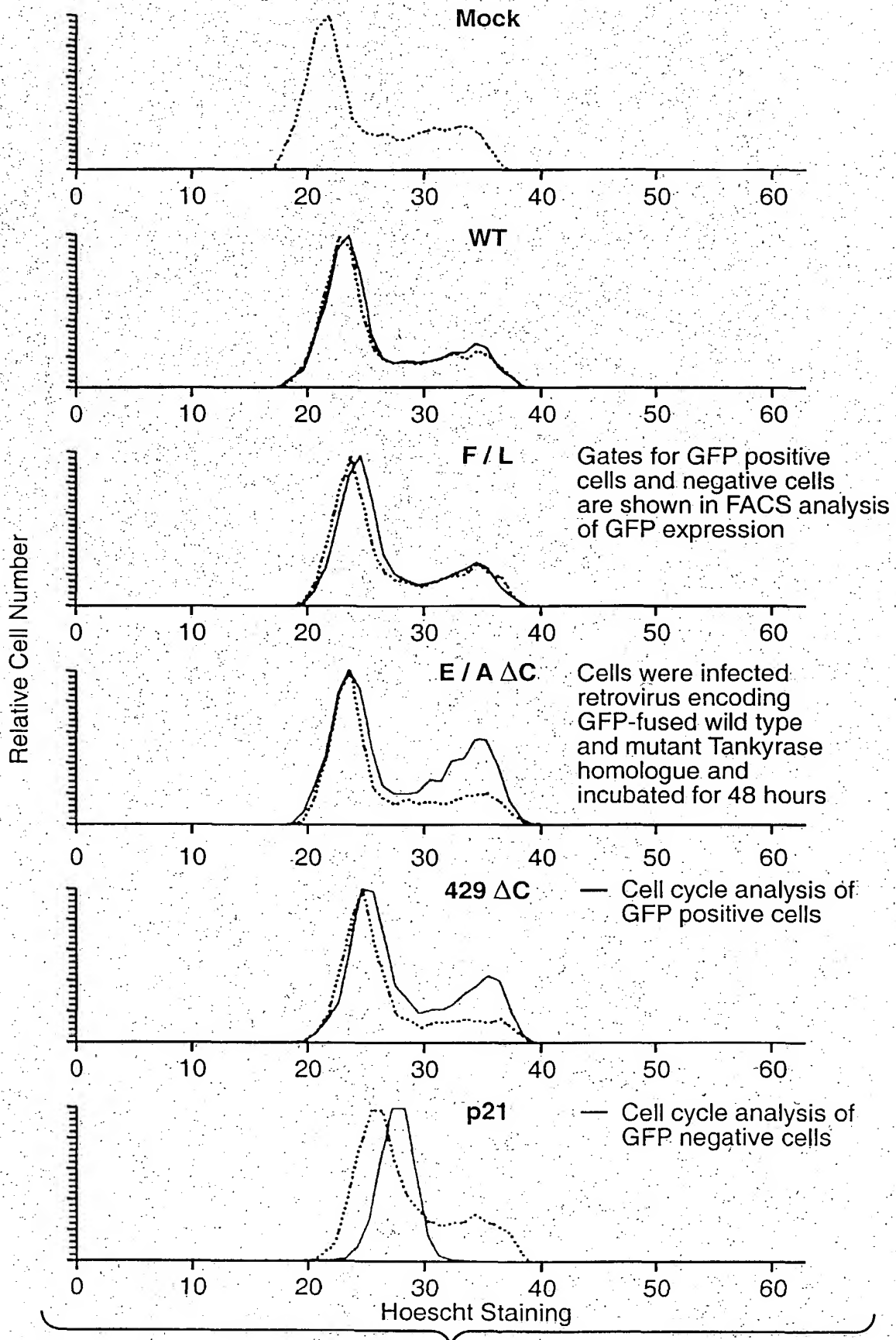

Mock
WT
F / L
E / A ΔC
429 ΔC
p21
0
10
20
30
40
50
60
Relative Cell Number
Hoescht Staining
Gates for GFP positive cells and negative cells are shown in FACS analysis of GFP expression
Cells were infected retrovirus encoding GFP-fused wild type and mutant Tankyrase homologue and incubated for 48 hours
— Cell cycle analysis of GFP positive cells
— Cell cycle analysis of GFP negative cells

FIG. 6B

Kinetics of GFP Positive cells in A549 Cells and Human Mammary Epithelial Cells (HMEC) After Retrovirus Infection Encoding GFP-fused Wild Type and Mutant Tankyrase Homologue

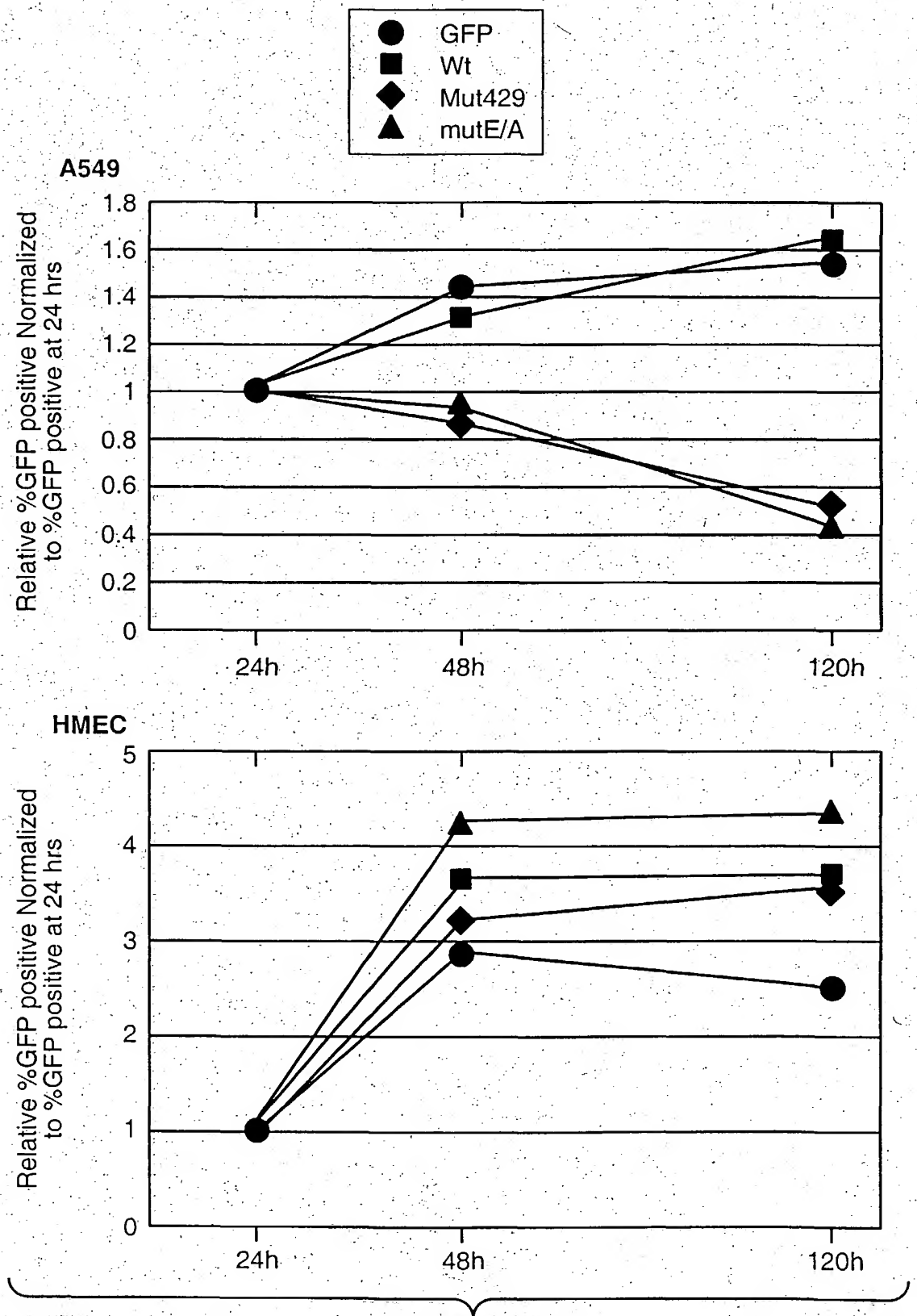
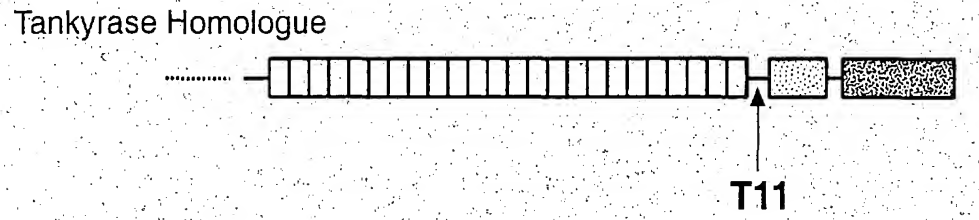


FIG. 7

The Binding Site of Antisense Oligos Against Tankyrase Homologue



Ankyrin rpeat SAM PARP

Unidentified sequence

```
                    T11
                    GTGGAACAGAGGGTGCTTCC
Tankyrase Homologue GTGGAACAGAGGGTGCTTCCAGTTTGGAGAAAAAGGAGGTTCCAGGAGTAGATTTTAGCAT 2838
Tankyrase           ATGCAGGGGATGGCGCCGCGGGAACAGAAAGGAAGGAAGGAGAAGTTGCTGGTCTTGACAT 3091
                     ** *   ** ** **  *  *   ** *  ***** *    **  *  * * **  ***
```

FIG._8

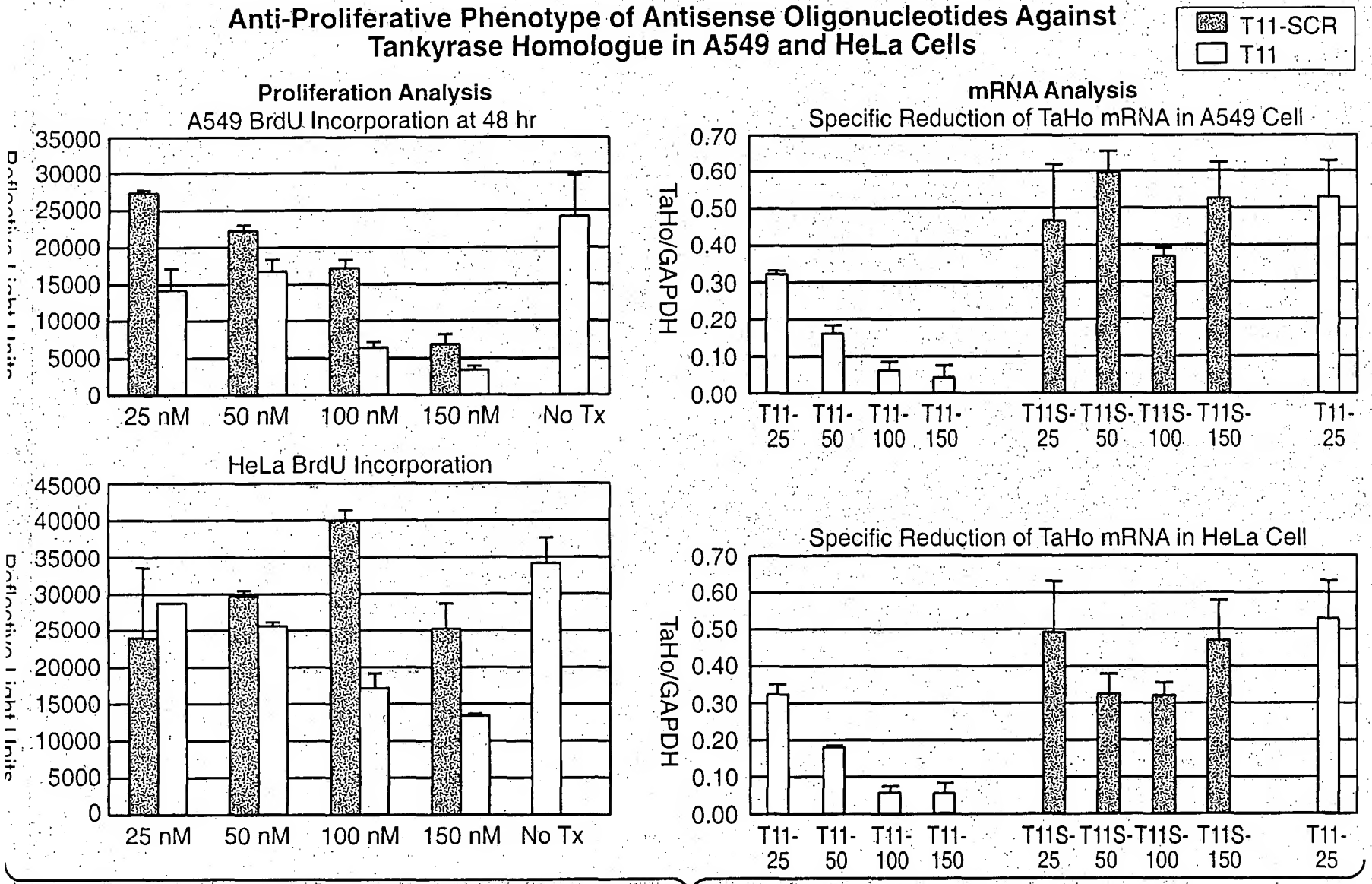

Anti-Proliferative Phenotype of Antisense Oligonucleotides Against Tankyrase Homologue in A549 and HeLa Cells
T11-SCR
T11
Proliferation Analysis
A549 BrdU Incorporation at 48 hr
Deflection Light Units
35000
30000
25000
20000
15000
10000
5000
0
25 nM
50 nM
100 nM
150 nM
No Tx
HeLa BrdU Incorporation
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
mRNA Analysis
Specific Reduction of TaHo mRNA in A549 Cell
TaHo/GAPDH
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
T11-25
T11-50
T11-100
T11-150
T11S-25
T11S-50
T11S-100
T11S-150
T11-25
Specific Reduction of TaHo mRNA in HeLa Cell

FIG._9

Cell Cycle Analysis of A549 Cells Transfected with Antisense Oligonucleotides Against Tankyrase Homologue at 48 Hours, Antisense Oligonucleotides (T11) and Control Oligonucleotides (T11S) were transfected with FITC-labeled random 20mer Oligonucleotides (FITC), After 48 Hours, entire population (R1) and Top 5% (R2) of FITC transfected cells were analyzed for cell cycle

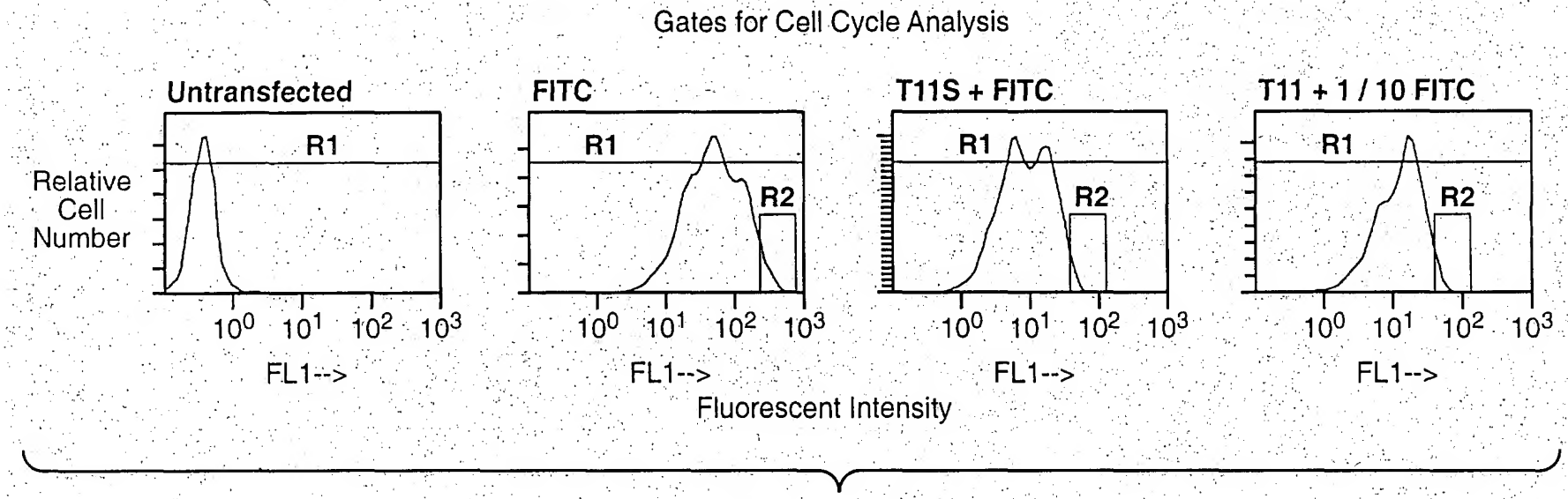
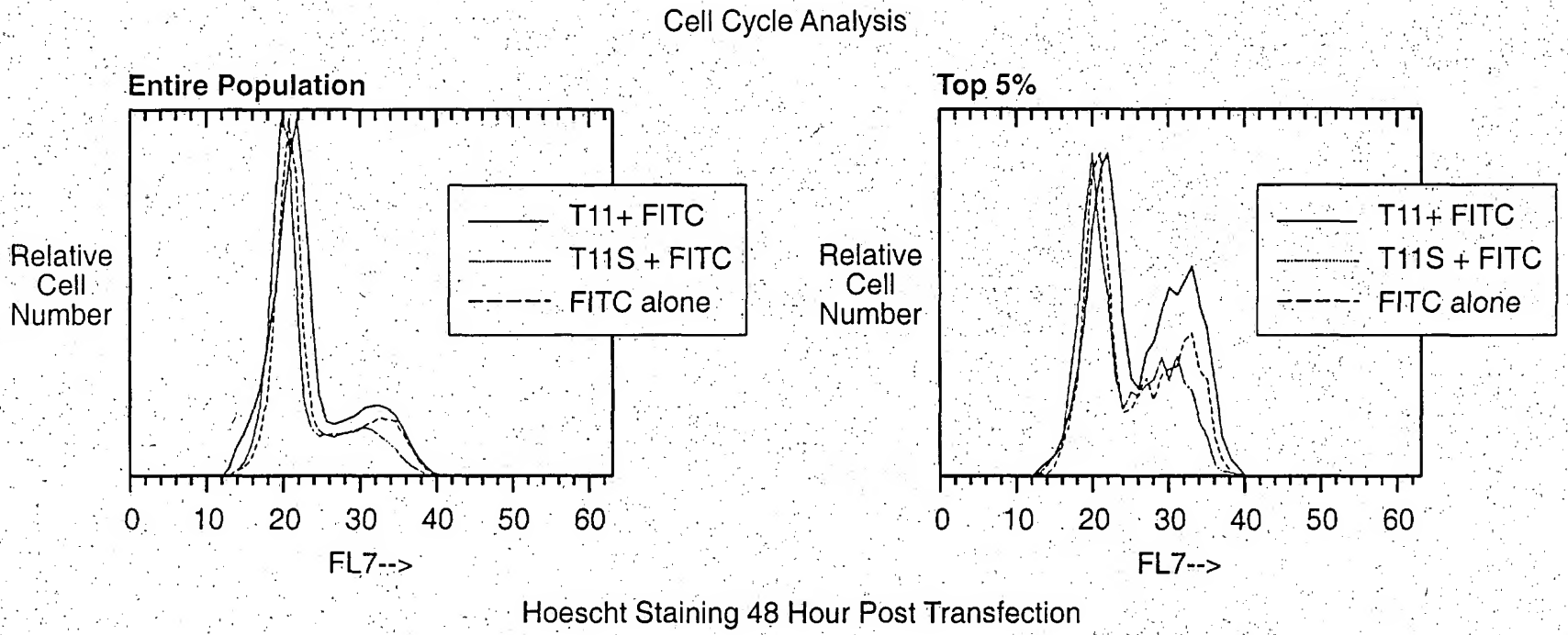


FIG._10A

Cell Cycle Analysis of A549 Cells Transfected with Antisense Oligonucleotides Against Tankyrase Homologue at 48 Hours, Antisense Oligonucleotides (T11) and Control Oligonucleotides (T11S) were transfected with FITC-labeled random 20mer Oligonucleotides (FITC), After 48 Hours, entire population (R1) and Top 5% (R2) of FITC transfected cells were analyzed for cell cycle



Hoescht Staining 48 Hour Post Transfection

FIG._10B

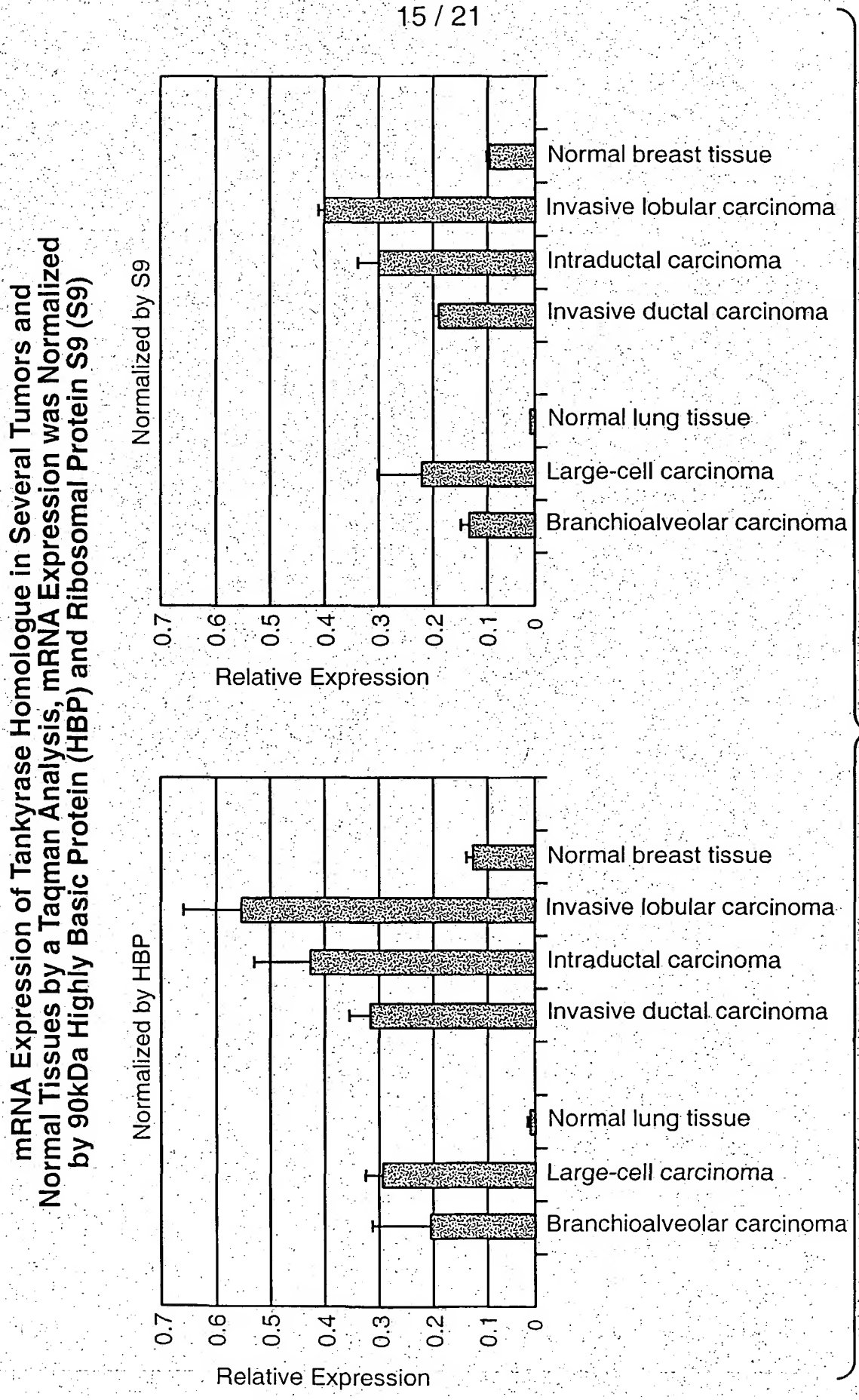

mRNA Expression of Tankyrase Homologue in Several Tumors and Normal Tissues by a Taqman Analysis, mRNA Expression was Normalized by 90kDa Highly Basic Protein (HBP) and Ribosomal Protein S9 (S9)
Normalized by S9
Normal breast tissue
Invasive lobular carcinoma
Intraductal carcinoma
Invasive ductal carcinoma
Normal lung tissue
Large-cell carcinoma
Branchioalveolar carcinoma
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Relative Expression
Normalized by HBP
Normal breast tissue
Invasive lobular carcinoma
Intraductal carcinoma
Invasive ductal carcinoma
Normal lung tissue
Large-cell carcinoma
Branchioalveolar carcinoma
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Relative Expression

FIG._11

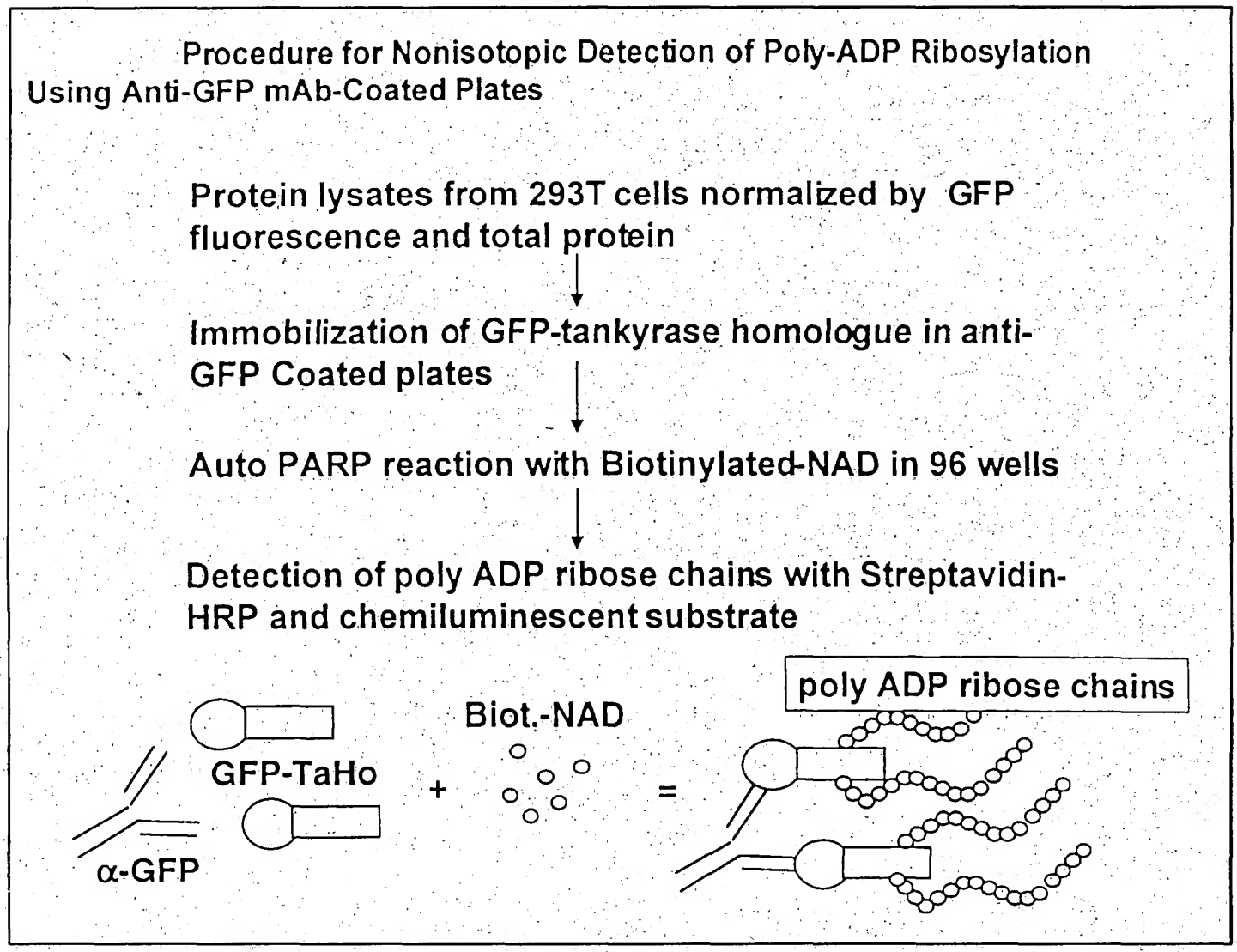

Procedure for Nonisotopic Detection of Poly-ADP Ribosylation Using Anti-GFP mAb-Coated Plates
Protein lysates from 293T cells normalized by GFP fluorescence and total protein
Immobilization of GFP-tankyrase homologue in anti-GFP Coated plates
Auto PARP reaction with Biotinylated-NAD in 96 wells
Detection of poly ADP ribose chains with Streptavidin-HRP and chemiluminescent substrate
GFP-TaHo
α-GFP
+
Biot.-NAD
=
poly ADP ribose chains

FIG._12

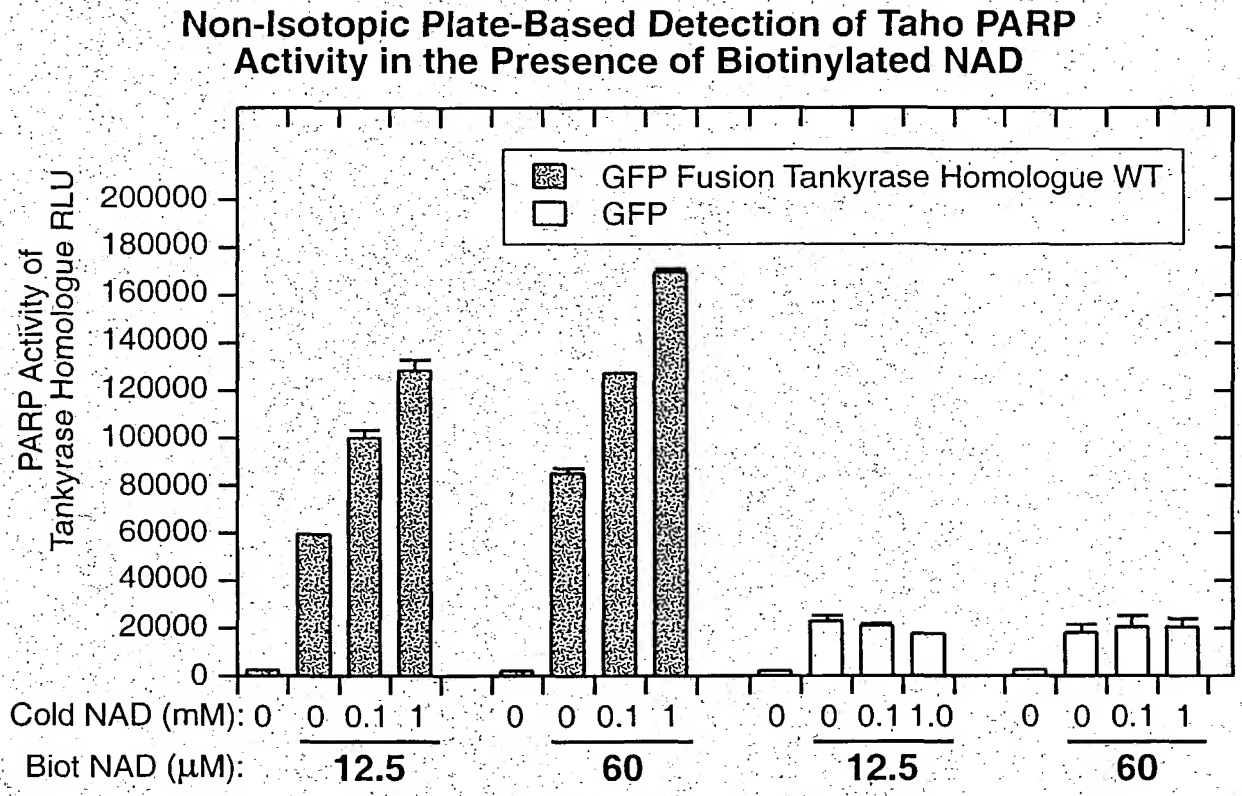


FIG._13

Comparison of IC_{50} Values of the PARP Inhibitors

	Approximate IC_{50} (nM)	hPARP assay IC_{50} (nM)		
	TaHo	Rigel	Decker*	Rankin*
3AB	>50 000	5 000	2 000	5 400
6(5H)Phenanthridinone	1 000-2 000	300		
Niacinamide	>50 000	30 000	>>5 000	31 000

* Decker P et al., Clinical Cancer Research. 1999 May; 5:1169-1172
* Rawkin PW et al., J Biol Chem. 1989 Mar 15;264(8):4312-4317

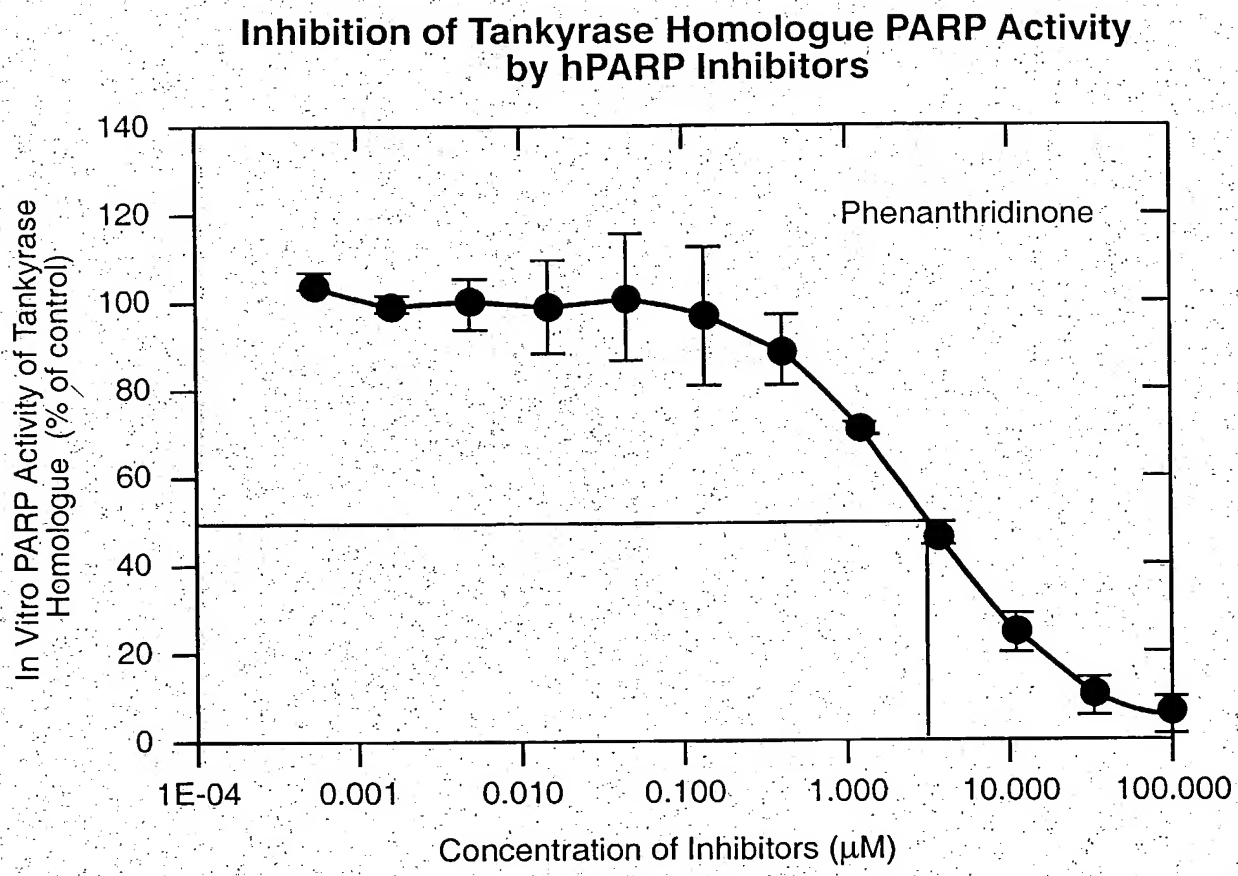


FIG._15

FIG._16

FIG._16A

FIG._16B

FIG._16C

'H–1: Tankyrase Homologue isoform–1, TH–2: Tankyrase Homologue isoform–2
/I (Red): the first methionine in the sequence, Z: stop codon
n this figure, the first methyonine in TH–1 sequence is position 1 (M1)

FIG._16A

aho C terminus deletion mutant ends at position 429 (K) and adds 28 amino acids because
f frame shift.

aho F/L mutant has the mutation at position 871

aho E/A dC mutant has the mutation at position 948, ends at position 957 (A) and adds 2
mino acids.

```
H-1                ------------------------------------------------------------
H-2                                        RCSARRGAAGGQGAQRGARVGAAHGTAPDPVTAGSQ -231

H-1                ------------------------------------------------------------
H-2                AARALSASSPGGLALLLAGPGLLLRLLALLLAVAAARIMSGRRCAGGGAACASAAAEAVE -171

H-1                ------------------------------------------*GFGRKDVVEYLLQNGA -111
H-2                PAARELFEACRNGDVERVKRLVTPEKVNSRDTAGRKSTPLHFAAGFGRKDVVEYLLQNGA -111
                            Ankyrin repeat                    Ankyrin repeat

H-1                SVQARDDGGLIPLHNACSFGHAEVVNLLLRHGADPNARDNWNYTPLHEAAIKGKIDVCIV -51
H-2                NVQARDDGGLIPLHNACSFGHAEVVNLLLRHGADPNARDNWNYTPLHEAAIKGKIDVCIV -51
                        Ankyrin repeat                    Ankyrin repeat
                                                                •TH1 start
H-1                LLQHGAEPTIRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSGNEEKMMALLTPLNV 10
H-2                LLQHGAEPTIRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSGNEEKMMALLTPLNV 10
```

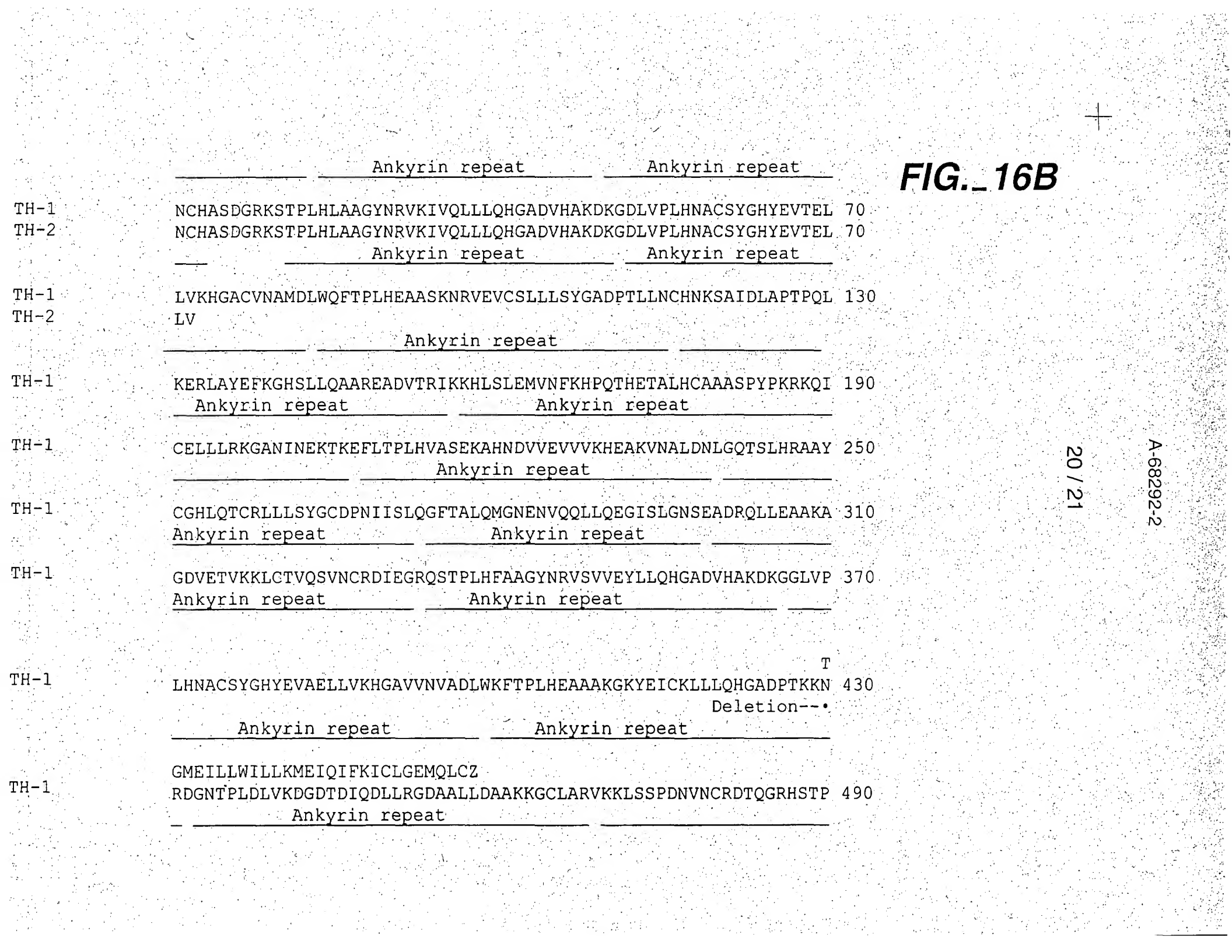

Ankyrin repeat
Ankyrin repeat
TH-1 NCHASDGRKSTPLHLAAGYNRVKIVQLLLQHGADVHAKDKGDLVPLHNACSYGHYEVTEL 70
TH-2 NCHASDGRKSTPLHLAAGYNRVKIVQLLLQHGADVHAKDKGDLVPLHNACSYGHYEVTEL 70
Ankyrin repeat
Ankyrin repeat
TH-1 LVKHGACVNAMDLWQFTPLHEAASKNRVEVCSLLLSYGADPTLLNCHNKSAIDLAPTPQL 130
TH-2 LV
Ankyrin repeat
TH-1 KERLAYEFKGHSLLQAAREADVTRIKKHLSLEMVNFKHPQTHETALHCAAASPYPKRKQI 190
Ankyrin repeat
Ankyrin repeat
TH-1 CELLLRKGANINEKTKEFLTPLHVASEKAHNDVVEVVVKHEAKVNALDNLGQTSLHRAAY 250
Ankyrin repeat
TH-1 CGHLQTCRLLLSYGCDPNIISLQGFTALQMGNENVQQLLQEGISLGNSEADRQLLEAAKA 310
Ankyrin repeat
Ankyrin repeat
TH-1 GDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYNRVSVVEYLLQHGADVHAKDKGGLVP 370
Ankyrin repeat
Ankyrin repeat
T
TH-1 LHNACSYGHYEVAELLVKHGAVVNVADLWKFTPLHEAAAKGKYEICKLLLQHGADPTKKN 430
Deletion--•
Ankyrin repeat
Ankyrin repeat
GMEILLWILLKMEIQIFKICLGEMQLCZ
TH-1 RDGNTPLDLVKDGDTDIQDLLRGDAALLDAAKKGCLARVKKLSSPDNVNCRDTQGRHSTP 490
Ankyrin repeat

FIG._16B

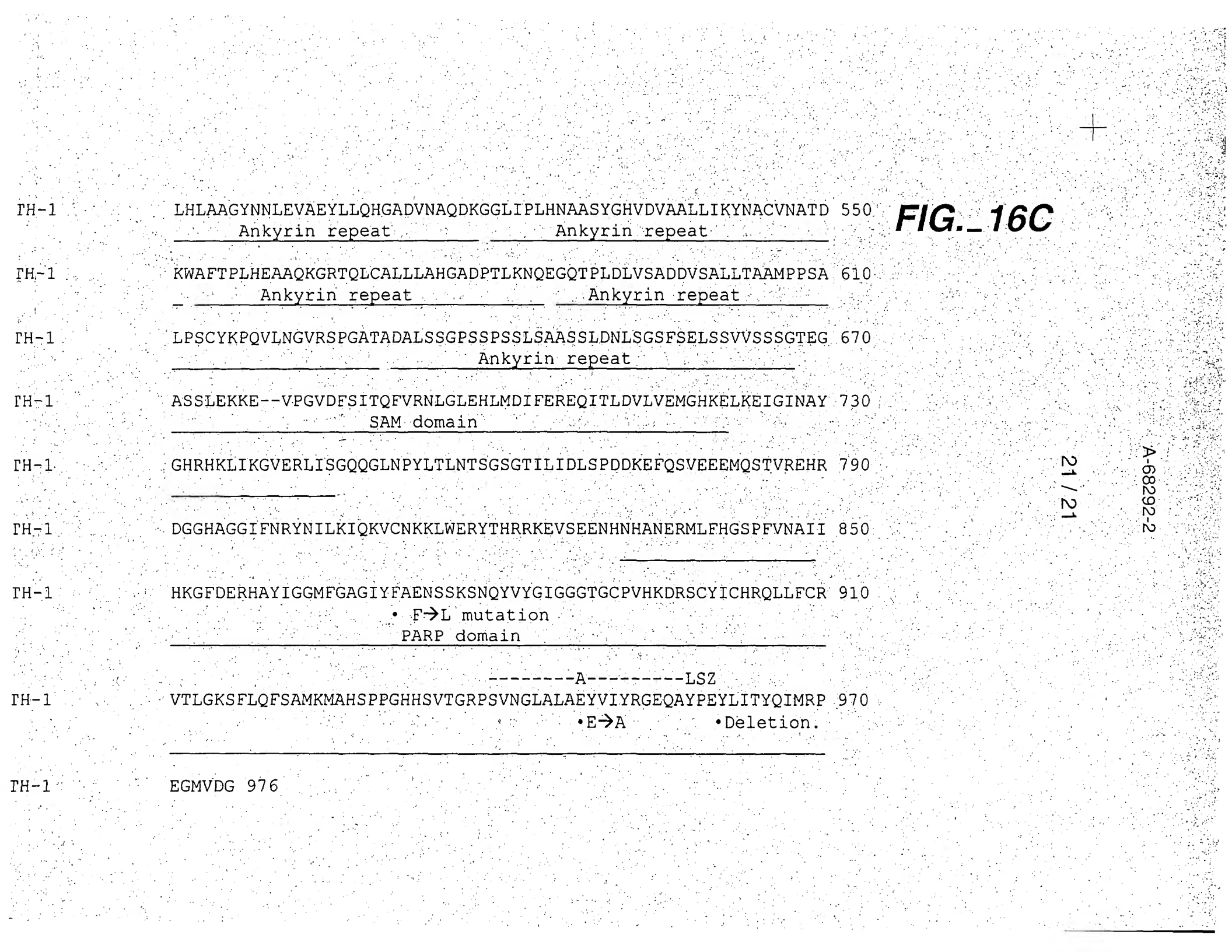

TH-1 LHLAAGYNNLEVAEYLLQHGADVNAQDKGGLIPLHNAASYGHVDVAALLIKYNACVNATD 550
Ankyrin repeat
Ankyrin repeat
TH-1 KWAFTPLHEAAQKGRTQLCALLLAHGADPTLKNQEGQTPLDLVSADDVSALLTAAMPPSA 610
Ankyrin repeat
Ankyrin repeat
TH-1 LPSCYKPQVLNGVRSPGATADALSSGPSSPSSLSAASSLDNLSGSFSELSSVVSSSGTEG 670
Ankyrin repeat
TH-1 ASSLEKKE--VPGVDFSITQFVRNLGLEHLMDIFEREQITLDVLVEMGHKELKEIGINAY 730
SAM domain
TH-1 GHRHKLIKGVERLISGQQGLNPYLTLNTSGSGTILIDLSPDDKEFQSVEEEMQSTVREHR 790
TH-1 DGGHAGGIFNRYNILKIQKVCNKKLWERYTHRRKEVSEENHNHANERMLFHGSPFVNAII 850
TH-1 HKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPVHKDRSCYICHRQLLFCR 910
• F→L mutation
PARP domain
--------A---------LSZ
TH-1 VTLGKSFLQFSAMKMAHSPPGHHSVTGRPSVNGLALAEYVIYRGEQAYPEYLITYQIMRP 970
•E→A •Deletion.
TH-1 EGMVDG 976

FIG._16C